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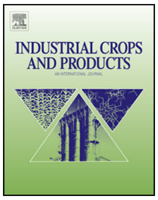

Optimization of antioxidants extraction from coffee silverskin, a roasting by-product, having in view a sustainable process

Anabela S.G. Costa[a], Rita C. Alves[a,b,*], Ana F. Vinha[a,c], Sérgio V.P. Barreira[c], Maria A. Nunes[a], Luís M. Cunha[d], M. Beatriz P.P. Oliveira[b]

[a] *REQUIMTE/Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal*
[b] *REQUIMTE/Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida, 431, 4200-072 Porto, Portugal*
[c] *FCS-UFP/Faculdade de Ciências da Saúde, Universidade Fernando Pessoa, Rua Carlos da Maia, 296, 4200-150 Porto, Portugal*
[d] *REQUIMTE/DGAOT, Faculdade de Ciências, Universidade do Porto, Rua Padre Armando Quintas, 7, Vairão, 4486-661 Vila do Conde, Portugal*

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ABSTRACT

Coffee silverskin is a major roasting by-product that could be valued as a source of antioxidant compounds. The effect of the major variables (solvent polarity, temperature and extraction time) affecting the extraction yields of bioactive compounds and antioxidant activity of silverskin extracts was evaluated. The extracts composition varied significantly with the extraction conditions used. A factorial experimental design showed that the use of a hydroalcoholic solvent (50%:50%) at 40 °C for 60 min is a sustainable option to maximize the extraction yield of bioactive compounds and the antioxidant capacity of extracts. Using this set of conditions it was possible to obtain extracts containing total phenolics (302.5 ± 7.1 mg GAE/L), tannins (0.43 ± 0.06 mg TAE/L), and flavonoids (83.0 ± 1.4 mg ECE/L), exhibiting DPPH$^{\bullet}$ scavenging activity (326.0 ± 5.7 mg TE/L) and ferric reducing antioxidant power (1791.9 ± 126.3 mg SFE/L). These conditions allowed, in comparison with other "more effective" for some individual parameters, a cost reduction, saving time and energy.

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1. Introduction

In recent years, sustainability has become an essential aspect to be observed in any human activity. For example, sustainable food manufacturing and processing (which reduces raw materials waste and minimizes refuse) is already a mandatory component of the business model. Furthermore, it can represent a challenge and an opportunity to innovate, looking for better commodities and production technologies, improving the overall environmental performance of products throughout their life-cycle, and, in this way, to gain the confidence of the increasingly informed consumers.

In the specific case of coffee producers, several procedures have already been adopted to obtain a more sustainable product namely: organic farming practices, preserving native forests and biodiversity, bird safe and shade grown coffee, and fair-trade coffee. With this, producers aimed to obtain a certification that will provide them preferential market access and sell at a differential price. Nevertheless, the coffee industry (the second largest commodity in the world after oil industry) still generates large amounts of residues representing serious environmental problems (Mussatto et al., 2011) and, because of that, waste management is also an increasingly important issue. Studies show that coffee husks, a residue from coffee processing, are potential raw materials for bioethanol production (Gouvea et al., 2009). Other alternative is biodiesel production using oil extracted from defective coffee beans instead of roasting for consumption of beverage with depreciated quality (Oliveira et al., 2008b). Other potential examples of by-products valorization in the coffee industry are the use of defective coffee beans press cake (resultant from biodiesel production) or coffee husks to be used as adsorbents for the removal of dyes from aqueous media (Oliveira et al., 2008a). The production of low-cost adsorbents from coffee husks for the treatment of wastewater containing heavy metals has been under study as well (Oliveira et al., 2008c).

The aforementioned recycling alternatives are only interesting for coffee producing countries while in importing countries, where roast of green processed beans is performed, the wastes are mainly associated with roasting and consumption: coffee silverskin and spent grounds, respectively. The last example consists in the remains of ground roasted coffee after beverage preparation. Recently, a lot of attention is being given to their recycling, namely,

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[*] Corresponding author at: REQUIMTE/Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida, 431, 4200-072 Porto, Portugal. Tel.: +351 22 834 05 37.
E-mail address: rita.c.alves@gmail.com (R.C. Alves).

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as a possible fertilizer after composting, a versatile barrier in reducing pesticide leaching through soil, a source of oil for biodiesel or biomass for bunker fuel substitution (Adi and Noor, 2009; Fenoll et al., 2011; Kondamudi et al., 2008).

Coffee silverskin is an integument that covers the raw coffee bean. This light pellicle constitutes a by-product of coffee roasting since it is detached during this procedure. Since usually it is not used to prepare coffee beverages, silverskin is separated from the beans by air flow and used, afterwards, as firelighters or dispatched for landfills.

The preparation of dietary supplements/nutraceuticals, food ingredients, and some pharmaceutical products is increasingly made from the extraction of bioactive compounds from natural products (Dai and Mumper, 2010). We believe that coffee silverskin might be an important source of several bioactive compounds. In fact, the scarce literature about this matrix suggests that it contains dietary fiber (Napolitano et al., 2007; Pourfarzad et al., 2013) and antioxidants (Borrelli et al., 2004; Murthy and Naidu, 2012), two recognized factors in the prevention of chronic diseases (Kris-Etherton et al., 2002). In the work presented herein, we aimed to optimize a sustainable method to obtain silverskin extracts with the highest possible amount of antioxidants. The effect of the main variables affecting the extraction yields and antioxidant activity of extracts were studied, namely, solvent polarity, temperature and time of extraction. Water and ethanol were selected because of their low toxicity. In order to modulate the solvent polarity, different ratio mixtures (25:75, 50:50 and 75:25) were used. An experimental design for analyzing the effects of time and temperature of extraction was performed for each solvent mixture. Extracts were compared regarding their total phenolics, flavonoids and tannins contents. Moreover, their antioxidant capacity was assessed by two complementary procedures, namely, the ferric reducing antioxidant power (FRAP) method and the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH$^{\bullet}$) scavenging capacity assay.

With this, we aim to contribute to coffee silverskin valorization and recycling, taking advantage of its potential health properties since its extracts could be henceforth applied in products such as antioxidant food supplements or cosmetic products.

2. Materials and methods

2.1. Reagents and standards

Gallic acid, tannic acid, epicatechin, trolox, sodium acetate, Folin–Ciocalteu's phenol reagent, DPPH$^{\bullet}$ (2, 2-diphenyl-1-picrylhydrazyl), sodium nitrite, ferric chloride, aluminum chloride, TPTZ (2,4,6-tripyridyl-s-triazine) solution, and ferrous sulfate heptahydrate were all obtained from Sigma–Aldrich (St. Louis, USA). Sodium carbonate anhydrous, sodium hydroxide and absolute ethanol were purchased from Merck (Darmstadt, Germany). Ultrapure water was treated in a Milli-Q water purification system (Millipore, Bedford, MA, USA) and used to prepare all aqueous solutions.

2.2. Samples and sample preparation

Silverskin sample were gently provided by a national coffee roaster industry (Bicafé – Torrefação e Comércio de Café, Lda., Portugal). Sample was representative of the major by-product of this industry and resulted from the roast of a commercial coffee blend constituted by ~40% of arabica and ~60% of robusta coffee beans (*Coffea arabica* and *Coffea canephora* var. *robusta*, respectively). After reception, samples were ground (Grindomix GM 200, Retsch, Haan, Germany), homogenized and used to prepare extracts.

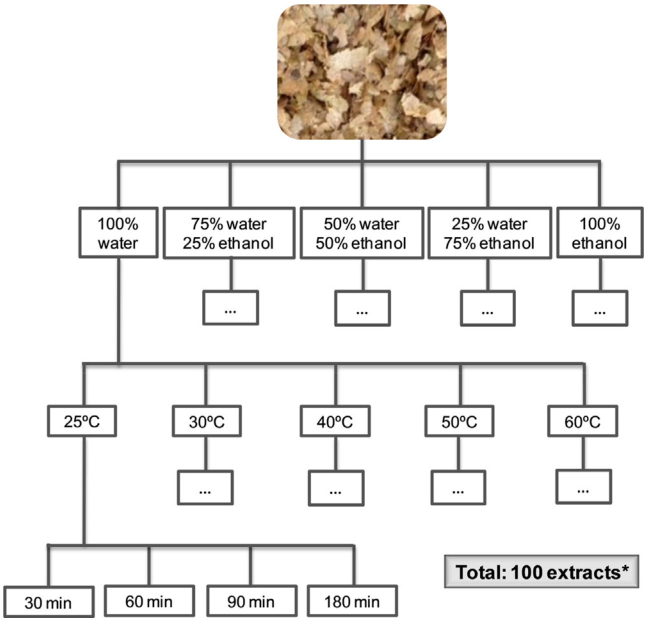

Fig. 1. Scheme of the silverskin extracts preparation. *Each extract was prepared in triplicate and each replicate was analyzed in triplicate.

2.3. Extracts preparation

In order to study the optimal extraction conditions, different procedures were tested by varying solvent polarity, temperature, and time of extraction. Fig. 1 outlines the different conditions used to prepare extracts.

Briefly, 1 g of ground sample and 50 mL of solvent/solvent mixture (100% water, 75% water/25% ethanol, 50% water/50% ethanol, 25% water/75% ethanol or 100% ethanol) were brought into contact at different temperatures (25, 30, 40, 50, and 60 °C), for periods of 30, 60, 90, and 180 min. Extractions were performed on a heating plate with constant stirring (600 rpm) and each set of conditions (solvent, time and temperature) was performed in triplicate. The final extracts were filtered and stored at −25 °C prior analysis.

2.4. Total phenolics content

Total phenolics contents of diluted extracts (1:10) were determined according to Alves et al. (2010). Briefly, 500 μL of each extract were mixed with 2.5 mL of the Folin–Ciocalteu reagent (1:10) and 2 mL of a sodium carbonate solution (7.5% m/v). The mixture was first incubated at 45 °C, during 15 min, followed by 30 min incubation at room temperature before absorbance readings at 765 nm were performed. Total phenolics content was calculated from a calibration curve prepared with gallic acid (10–100 mg/L; $r = 0.9997$) and expressed as mg of gallic acid equivalents (GAE)/L of extract.

2.5. Total flavonoids content

Total flavonoids contents were determined according to Barroso et al. (2011) with slight modifications. Aliquots of 1 mL of extract were mixed with 4 mL of distilled water and 300 μL of 25% sodium nitrite. After 5 min at room temperature, 300 μL of 10% $AlCl_3$ were added, and 1 min after 2 mL sodium hydroxide (1 M) and 2.4 mL of ultrapure water. The absorbance was recorded at 510 nm. Total flavonoids content was calculated through a calibration curve of epicatechin (50–450 mg/L; $r = 0.9998$) and expressed as mg of epicatechin equivalents (ECE)/L of extract.

2.6. Total tannins content

Total tannins contents were determined according to Shad et al. (2012), with slight modifications. Briefly, 500 μL of extract (diluted at 1:10 when necessary) were mixed with 2.5 mL of the Folin–Ciocalteu reagent (1:10). After 3 min, 2 mL of sodium carbonate (7.5% m/v) were added. The mixture was kept in the dark for 2 h. Absorbance readings were carried out at 725 nm. Tannins content was calculated from a calibration curve of tannic acid (0–0.2 mg/L; $r = 0.9999$) and expressed as mg of tannic acid equivalents (TAE)/L of extract.

2.7. Antioxidant activity

2.7.1. DPPH• scavenging activity

The radical scavenging ability of extracts was analyzed according to the method described by Barroso et al. (2011) with some modifications. Briefly, 14 μL of diluted extract (1:10) were mixed with 186 μL of a freshly prepared DPPH• solution (9.3×10^{-5} mol/L in ethanol). The decrease of the DPPH• was measured in equal time intervals of 10 min by monitoring the decrease of absorption at 525 min, in order to observe the kinetics reaction. The reaction endpoint was attained in 40 min. A calibration curve was prepared with trolox (25–175 mg/L, $r = 0.9995$) and DPPH• scavenging activity was expressed as mg of trolox equivalents (TE)/L of extract.

2.7.2. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed according to Benzie and Strain (1996) with slight modifications. Briefly, 90 μL of diluted extract (1:10) were mixed with 270 μL of distilled water and 2.7 mL of the FRAP solution (containing 0.3 M acetate buffer, 10 mM TPTZ solution, and 20 mM of ferric chloride). After homogenization, the mixture was kept for 30 min at 37 °C protected from light. Absorbance was measured at 595 nm. A calibration curve was prepared with ferrous sulfate (50–450 mg/L, $r = 0.9998$) and ferric reducing antioxidant power was expressed as mg of ferrous sulfate equivalents (FSE)/L of extract.

2.7.3. Statistical analysis

A factorial experimental design (5 × 5 × 4) was used to evaluate the influence of the variables solvent, temperature and time of extraction on the dependent variables: phenolics, flavonoids, tannins, DPPH• and FRAP. The dependent variables were measured thrice for each set of extraction operating conditions (solvent, temperature, time). The set of vectors (solvent, temperature, time) were grouped into homogeneous groups through a hierarchical Cluster analysis by the method of least distance (Nearest Neighbor) using the squared Euclidean distance as the measure of dissimilarity. The Clusters were compared using a MANOVA analysis after validating the assumptions of multivariate normality and homogeneity of variance–covariance. Whenever the MANOVA analysis detected statistically significant effects, an ANOVA, for each of the dependent variables, was performed followed by Tukey's HSD post hoc test. The Multiple Linear Regression procedure was used to obtain a model that would allow predicting the value of each dependent variable as a function of operating conditions of extraction (water concentration in the solvent, temperature, contact time). Pearson correlation tests were used to ascertain the existence of linear relationships between the contents of bioactive compounds and antioxidant activity. It was considered a 0.05 significance level for all tests. All statistical analyzes were performed with SPSS statistics (v21, IBM SPSS, Chicago, IL).

3. Results and discussion

The extraction yields of bioactive compounds from natural matrices depends highly on several factors (Narita and Inouye, 2012; Pinelo et al., 2004, 2005). Some important variables such as type of solvent, solvent-to-solid ratio, time, temperature, or pressure can be managed in order to optimize compounds extraction. Nevertheless, the role of each of these variables in the mass transfer kinetics of the process is not always obvious (Pinelo et al., 2005). The compounds of interest and the chemical composition of the matrix will then define the best conditions to achieve a maximum yield of extraction.

In this work, as previously described, pure solvents (water (w) or ethanol (e)) or hydroalcoholic mixtures (25%w:75%e, 50%w:50%e, 75%w:25%e) were tested, together with different times and temperatures in an experimental factorial design set up. Total phenolics, flavonoids, and tannins contents, as well as antioxidant activity (using both DPPH• inhibition and FRAP assays) of the obtained extracts were evaluated. Results are depicted in Figs. 2 and 3, respectively.

As can be observed in Fig. 2, the set of conditions used highly influenced the extracts composition. Some of the resulting extracts are considerably richer in phenolic compounds (>310 mg GAE/L). That is the case of those obtained using a hydroalcoholic mixture (50%w:50%e), at 50 °C or 60 °C, during 90 min. On the other hand, 25%w:75%e/60 °C/90 min was the set of conditions that allowed a significantly higher extraction of flavonoids (144 mg ECE/L). The highest tannins concentration (0.43 mg TAE/L) was obtained using 50%w:50%e/40 °C/60 min and 50%w:50%e/60 °C/180 min. Although tannins ingestion has been related with anti-nutritional effects (since they form complexes with proteins, starch, and digestive enzymes causing a reduction in the nutritional value of foods), their antioxidant, anti-mutagenic and antimicrobial properties are also well documented, depending on the amount and type of tannins present in the food (Chung et al., 1998). In any case, the maximum total tannins content found in our extracts (~0.40 mg TAE/L) is very low when compared with other matrices (e.g. 41–64 mg/L for green/black tea (Lima et al., 2012)).

The worst extracting solvent for total phenolics was pure ethanol, followed by 100% water. Both options were also bad choices to extract flavonoids, as expected, due to the intermediate polarity of these compounds (Bravo, 1998). Curiously, for tannins, when extraction was carried out at ambient temperature (25 °C), ethanol was the best extracting solvent (being 25%w:75%e the worst). Even so, never achieved the extraction yield obtained with 50w:50e/40 °C/60 min or 50w:50e/60 °C/180 min. For the remaining temperatures/times of extraction, pure ethanol, followed by 100% water, were the worst solvents.

The best yields obtained for total phenolics in this study were ~3-fold higher than those reported by Narita and Inouye (2012) for silverskin extracts using subcritical water (240 °C, 3.2 MPa): 130 mg GAE/g of extract. This was most likely due to the different solid-solvent ratios as well as the different extraction conditions used. Although Narita and Inouye (2012) also started from 1 g of coffee silverskin in 50 mL of solvent (100% water), they freeze-dried the resultant extract and prepared a new 1.0 mg/mL extract for total phenolics analysis. Therefore, our extracts were more concentrated. However, when comparing phenolics amounts per g of coffee silverskin, Narita and Inouye (2012) achieved superior yields using subcritical conditions (35 mg GAE/g of coffee silverskin against 16 mg GAE/g in our study, for our best set of conditions). Nevertheless, the use of subcritical water needs special equipment that is not always available in laboratories or small industries. That is why, in this work, we optimized the antioxidants extraction using different solvents, times and temperatures of extraction at normal conditions of pressure. When comparing 100% water

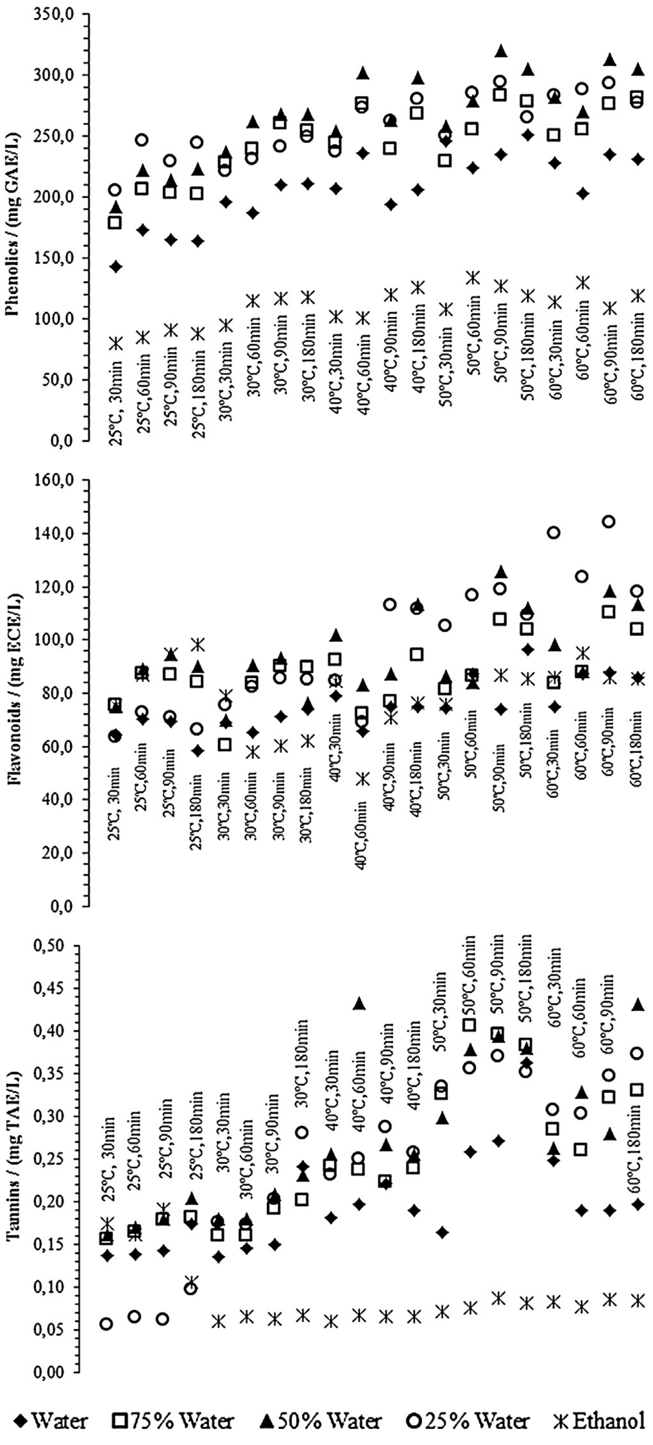

Fig. 2. Effect of solvent, time, and temperature in the extraction yield of total phenolics, flavonoids and tannins contents of the silverskin extracts. ◆water, □ 75% water, ▲ 50% water, ○ 25% water, ✳ ethanol.

extracts prepared in this work with those of Narita and Inouye (2012) obtained at 25 and 80 °C, during 1 h at normal conditions of pressure (6–7 mg GAE/g of coffee silverskin), we found that the extraction yield could be considerably improved by using a temperature of 50 °C for 30 min (~12 mg GAE/g of coffee silverskin). Nevertheless, Fig. 2 clearly shows that, for the conditions tested in this study, after pure ethanol, 100% water is the worst solvent to extract silverskin bioactive compounds, with the 50w:50e and 25w:75e hydroalcoholic mixtures giving the best results, a fact that is certainly linked with the polarity of the analyzed compounds (Bravo, 1998).

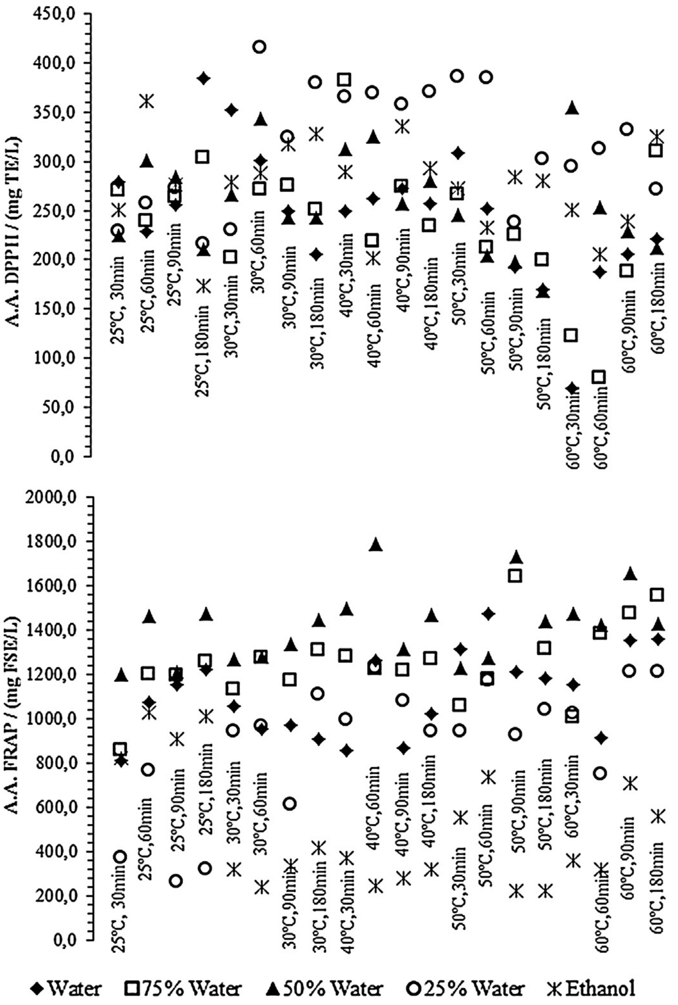

Fig. 3. Effect of solvent, time, and temperature in the antioxidant activity (A.A.) of the silverskin extracts. ◆water, □ 75% water, ▲ 50% water, ○ 25% water, ✳ ethanol.

As depicted in Fig. 3, and in accordance with the variations observed for bioactive compounds contents, the extraction conditions also affected significantly the antioxidant capacity of the extracts. Two assays were performed in order to evaluate different and complementary mechanisms of antioxidant action: ferric reducing antioxidant power (FRAP) and DPPH$^{\bullet}$ scavenging ability. In the FRAP assay, the reduction of ferric 2,4,6-tripyridyl-s-triazine (TPTZ) to a colored product is spectrophotometrically measured. This reaction detects compounds with redox potentials of <0.7 V (the redox potential of Fe^{3+}-TPTZ), being a reasonable screen for the ability to maintain redox status in cells or tissues. The FRAP mechanism is totally electron transfer, therefore this method cannot detect compounds that act by radical quenching (H transfer). On the other hand, in the DPPH$^{\bullet}$ scavenging assay, the radical may be neutralized either by direct reduction via electron transfers or by radical quenching via H atom transfer. This method is based on the measurement of the reducing ability of antioxidants toward DPPH$^{\bullet}$ which can be evaluated by measuring the decrease of its absorbance (Prior et al., 2005).

The extract with the highest DPPH$^{\bullet}$ scavenging ability (416 ± 4.2 mg ET/L) was obtained using the following conditions: 25%w:75%e/30 °C/60 min. In a general way, the best results for DPPH$^{\bullet}$ inhibition were achieved with this hydroalcoholic mixture, as was also previously observed for flavonoids contents, although no correlation was found between results of both parameters ($r = 0.048$). In what concerns to the FRAP assay, values exceeding 1700 mg SFE/L were found in the extracts obtained with 50%w:50%e/40 °C/60 min and 50%w:50%e/50 °C/90 min. These conditions were also among the best options to extract total phenolics and tannins. On the other hand, pure ethanol lead to inferior values in the FRAP assay. A similar behavior was observed for tannins: when extraction was carried out at ambient temperature (25 °C), 25%w:75%e was the worst extracting solvent while for the remaining temperatures/times of extraction, pure ethanol provided the lowest results. Nevertheless, considering all the results together, FRAP levels were more correlated with total phenolics ($r = 0.688$) and tannins ($r = 0.686$) than with flavonoids ($r = 0.376$).

In what concerns to the temperature of extraction, its increase for a given solvent/contact time improved the extraction yield of total phenolics and flavonoids, and increased the reducing power of the extract. In the case of tannins, this tendency was reversed at temperatures above 50 °C. The DPPH$^{\bullet}$ scavenging activity was the only parameter that decreased with higher temperatures of extraction. As no correlation was observed between the results of the DPPH$^{\bullet}$ assay and the contents of the analyzed compounds ($r = -0.053$, 0.048, and -0.083, for total phenolics, flavonoids, and tannins, respectively), other compounds present in coffee silverskin should be responsible for the antiradical activity of the extracts, namely melanoidins. Indeed, these compounds (which are Maillard products generated during the coffee roast) have their antioxidant properties well characterized and have been identified not only in coffee beverages (Nunes and Coimbra, 2007), but also in coffee silverskin (Borrelli et al., 2004). Such lack of correlation was also previously observed for coffee beverages prepared from beans roasted at different degrees (Alves et al., 2010), where a decrease in phenolic contents (due to thermal degradation) was not tracked by DPPH$^{\bullet}$ assay values that were maintained or even increased, being suggested the involvement of melanoidins in this occurrence. Narita and Inouye (2012) also found that, at normal conditions of pressure, the increase of temperature did not improve the results in the DPPH assay. In fact, they found similar values when aqueous extracts were prepared at 25 °C and 80 °C (74–75 μmol TE/g of extract), obtaining an improvement of the extraction yield only at subcritical conditions. Regarding other matrices, some authors (Sasidharan and Menon, 2011; Chew et al., 2011) also reported that the extracting temperature had a negative effect on DPPH$^{\bullet}$ scavenging activity. Chew et al. (2011) described that although the recovery of phenolic compounds increased linearly with extraction temperature, the antioxidant capacities of extracts from *Centella asiatica* decreased when the extraction temperature was higher than 45 °C. Probably, the compounds extracted at higher temperatures have lower anti-radical capacity as compared to those extracted at lower temperatures.

The effect of contact time appeared to depend on temperature. It was generally noticed that, at room temperature and above 50 °C, the extraction time did not influence the levels of the bioactive compounds nor the antioxidant activity of the extracts, while for intermediate temperatures (30, 40 and 50 °C), an increase in the contact time tendentially improved the performance in all parameters (except for the reducing power, whose value did not seem to be affected by this operating variable). At higher extraction temperatures, the contact time had a negative impact on the DPPH$^{\bullet}$ scavenging ability of the extracts. It has been reported that an extended extraction time could lead to a higher oxygen exposure (Naczk and Shahidi, 2004; Chirinos et al., 2007). In this case, it is possible that some degree of oxidation on anti-radical compounds from silverskin may occur.

These trends are confirmed statistically by the results of a multivariate regression analysis (Table 1).

The regression models are all significant ($p < 0.001$) despite explaining always less than 40% of the total variance. *P*-values also indicate that the operating variable "time of extraction" is not a significant predictor in the case of the dependent variables: phenolics, DPPH$^{\bullet}$ inhibition and FRAP. The sign of the regression coefficients indicates that the reducing power of the extract increases with contact time and water concentration in the solvent mixture. On the other hand, flavonoids and the DPPH$^{\bullet}$ inhibition increases slightly with decreasing concentration of water in the extracting solvent mixture. Notice, however that pure ethanol was, in general, the worst extracting solvent for all the groups of compounds analyzed. Increasing the temperature of the extraction has a beneficial effect on the extract yield of phenolics (1.54 mg GAE/L/°C), flavonoids (0.72 mg GAE/L/°C) and FRAP (4.70 mg GAE/L/°C), but reduces the extract DPPH$^{\bullet}$ radical scavenging capacity in 1.22 mg GAE/L/°C). A Cluster analysis, considering as variables all the extract parameters, showed that the extraction operating conditions can be allocated into 8 groups according to the dendrogram presented in Fig. 4. As can be seen, most of the operating conditions vectors (solvent, temperature, contact time), where the extracting solvent was water are included in Cluster 1. In contrast, the Clusters 5, 6, and 8, which are situated on the opposite side of the dendrogram, comprise vectors of operating conditions wherein the solvent is ethanol or mostly ethanol. Therefore, it may be concluded that the variable solvent is determining in the yields of the several extract parameters, something that the graphical representation of the raw data presented in Figs. 2 and 3 and the multivariable regression analysis already indicated. Clusters 2, 3 and 4 are composed of vectors of intermediate operating conditions (75%w/25%e or 50%w/50%e, 40 °C < temperature < 60 °C, 30 < contact time < 90). Finally, Cluster 7 includes only one set of operating conditions (ethanol, 25 °C, 180 min.). These Clusters (2, 3, 4, and 7) are close in the dendrogram, suggesting that to obtain a similar overall performance with ethanol to that obtained with 50%w/50%e, it is required a considerable increase of the extraction time. Through a MANOVA analysis using the extract concentration of phenolics, flavonoids, tannins, and the two measures of antioxidant activity as dependent variables, it was concluded that these Clusters of operating conditions are statistically different (Pillai's trace = 1.103, $p < 0.001$, Observed power = 1) (Table 2).

It was noted that FRAP values varied more between Clusters, ranging from 305.9 (Cluster 5) to 1791.90 mg SFE/L (Cluster 4). The yield in phenolics also varied considerably from Cluster to Cluster. On the other hand, the flavonoids yield is the parameter for which differences among Clusters are less noticeable. Taking into account the yield in each of the dependent variables, the Clusters may be arranged as follows:

Phenolics:
Cluster 2 ∼ Cluster 4 > Cluster 3 > Cluster 6 > Cluster 1 ≫ Cluster 5 > Cluster 8 > Cluster 7
Flavonoid:
Cluster 2 > Cluster 7 > Cluster 1 ∼ Cluster 6 ∼ Cluster 3 = Cluster 4 ∼ Cluster 8 > Cluster 5
Tannins:
Cluster 4 > Cluster 2 > Cluster 3 > Cluster 1 > Cluster 6 ≫ Cluster 7 > Cluster 8 = Cluster 5
DPPH$^{\bullet}$ scavenging activity:
Cluster 4 ∼ Cluster 6 ≫ Cluster 8 > Cluster 5 ∼ Cluster 1 > Cluster 2 ≫ Cluster 7 > Cluster 3
FRAP:
Cluster 4 > Cluster 2 ≫ Cluster 1 > Cluster 7 ∼ Cluster 3 ≫ Cluster 6 > Cluster 8 ≫ Cluster 5

The operating conditions corresponding to Cluster 4 (50%w:50%e/40 °C/60 min) stand out in the extraction yield of

Table 1
Multivariable regression statistics.

Variable	Total phenolics (mg GAE/L) ($R^2 = 0.269$)			Flavonoids (mg ECE/L) ($R^2 = 0.298$)			Tannins (mg TAE/L) ($R^2 = 0.320$)		
	β	95% IC	p	β	95%z IC	p	β	95% IC	p
			<0.001			<0.001			<0.001
Constant	106.93	76.28 to 137.58	<0.001	55.33	46.60 to 64.06	<0.001	–	−0.052 to 0.042	0.834
Temperature/°C	1.54	0.94 to 2.14	<0.001	0.72	0.55 to 0.89	<0.001	0.004	0.003 to 0.005	<0.001
Time/min	0.12	−0.02 to 0.26	0.081	0.06	0.02 to 0.10	0.003	0.000	0.000 to 0.000	0.024
% Water	0.73	0.51 to 0.95	<0.001	−0.069	−0.13 to −0.01	0.029	0.001	0.001 to 0.001	<0.001

Variable	DPPH (mg TE/L) ($R^2 = 0.116$)			FRAP (mg SFE/L) ($R^2 = 0.333$)		
	β	95% IC	p	β	95% IC	p
			<0.001			<0.001
Constant	347.16	308.9 to 385.4	<0.001	475.04	290.59 to 659.48	<0.001
Temperature/°C	−1.22	−1.97 to −0.47	0.002	4.70	1.07 to 8.32	0.011
Time/min	−0.04	−0.21 to 0.13	0.632	0.58	−0.25 to 1.40	0.169
% Water	−0.54	−0.81 to −0.26	<0.001	6.29	4.98 to 7.60	<0.001

Independent variables: % water in extraction solvent, temperature and contact time. Dependent variables: extract antioxidant activity (DPPH and FRAP) and concentration of bioactive compounds (total phenolics, flavonoids and tannins).
R^2 is the coefficient of determination; β represents the regression coefficients; the p-values are associated with the hypothesis that the coefficients are not significantly different from zero. 95% IC stands for 95% confidence interval for the β's.

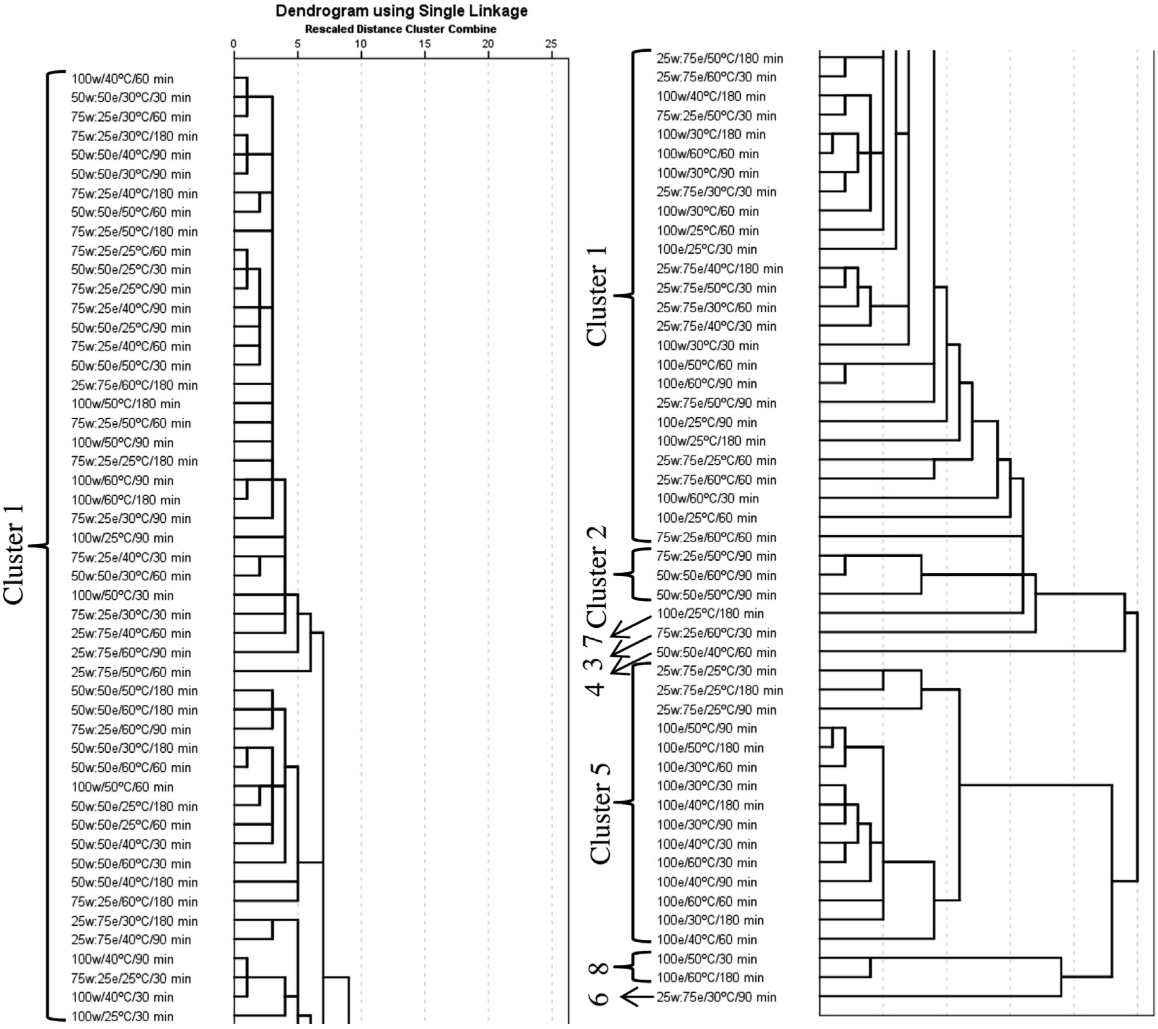

Fig. 4. Dendrogram resulting from a cluster analysis of the extraction operating conditions considering the bioactive compounds concentration in the extracts and their antioxidant capacity as variables.

Table 2
Mean values (± standard deviation) of the levels of bioactive compounds and antioxidant activity for the several Clusters of extraction conditions.

Cluster	Phenolics (mg GAE/L)	Flavonoids (mg ECE/L)	Tannins (mg TAE/L)	DPPH (mg TE/L)	FRAP (mg SFE/L)
1	$232.4 \pm 50.6^{a,b}$	$88.6 \pm 18.3^{a,b}$	$0.23 \pm 0.08^{b,c,d}$	$268.1 \pm 75.4^{a,b}$	$1161.2 \pm 239.1^{b,c}$
2	305.8 ± 18.1^{a}	117.3 ± 9.4^{a}	$0.36 \pm 0.06^{a,b}$	$217.0 \pm 27.8^{a,b}$	$1677.6 \pm 236.0^{a,b}$
3	$250.6 \pm 12.8^{a,b}$	$83.8 \pm 8.7^{a,b}$	$0.28 \pm 0.04^{a,b,c}$	121.5 ± 36.1^{b}	$1006.2 \pm 35.4^{c,d}$
4	302.5 ± 7.1^{a}	$83.0 \pm 1.4^{a,b}$	0.43 ± 0.06^{a}	326.0 ± 5.7^{a}	1791.9 ± 126.3^{a}
5	$137.5 \pm 46.8^{b,c}$	72.9 ± 13.5^{b}	0.07 ± 0.01^{d}	$271.5 \pm 55.6^{a,b}$	305.9 ± 78.7^{e}
6	$241.2 \pm 3.2^{a,b}$	$85.5 \pm 14.8^{a,b}$	$0.20 \pm 0.03^{b,c,d}$	324.5 ± 36.1^{a}	$609.7 \pm 242.5^{d,e}$
7	87.7 ± 1.4^{c}	$98.4 \pm 1.1^{a,b}$	$0.11 \pm 0.02^{c,d}$	$173.5 \pm 16.3^{a,b}$	$1013.3 \pm 75.7^{c,d}$
8	113.4 ± 7.1^{c}	$80.8 \pm 6.4^{a,b}$	0.08 ± 0.01^{d}	299.0 ± 49.2^{a}	$556.2 \pm 133.3^{d,e}$

In each column, values having at least one same superscript are not significantly different from each other ($p < 0.05$).

tannins and antioxidant activity. With regard to phenolic yield, are only surpassed by the conditions of Cluster 2 (75%w:25%e/50 °C/90 min; 50%w:50%e/60 °C/90 min; 50%w:50%e/50 °C/90 min). It is only in terms of flavonoids yield that the operating conditions corresponding to Cluster 4 are not among the best. The operating conditions that comprise the Cluster 2 are, after those of 4, the ones that provide better yields. In fact, they exceed the Cluster 4 conditions regarding the yield of flavonoids and phenolics. On the other hand, as previously mentioned, the set of operating conditions that are included in the Clusters 5 and 8 (mainly pure ethanol as solvent) were those that produce, in general, the worst yields. These differences should be mainly associated, as already discussed, with the polarity of the analyzed compounds.

4. Conclusion

Industrial waste production is a problem that affects the society in general. With regard to the coffee industry, several valorization procedures for different by-products are described in literature, nevertheless, the process sustainability is not always taken into consideration, and the options are often not viable from an energetic, economical or environmental point of view.

The present study aimed to select the optimal conditions for extracting antioxidant compounds from coffee silverskin, a main by-product of coffee torrefaction industry. The best set of conditions was variable according to the parameter analyzed (total phenolics, tannins, flavonoids or antioxidant capacity).

The highest phenolic content was obtained using a hydroalcoholic mixture (50%w:50%e), at 50 °C, during 90 min. On the other hand, 25%w:75%e/60 °C/90 min was the set of conditions that allowed a significantly higher extraction of flavonoids. The highest tannins concentration was attained with 50%w:50%e/40 °C/60 min. The extract with the highest DPPH$^{\bullet}$ scavenging ability was obtained using the following conditions: 25%w:75%e/30 °C/60 min. Regarding the FRAP values, the highest ones were achieved using 50%w:50%e/40 °C/60 min.

Nevertheless, in a sustainable perspective, it is possible to attain a compromise between the extraction conditions and the richness of the extract in antioxidants. Using a hydroalcoholic solvent (50%w:50%e), at 40 °C during 60 min, it is possible to obtain an extract rich in phenolic compounds, containing an average concentration of 302.5 ± 7.1 mg GAE/L total phenolics, 83.0 ± 1.4 mg ECE/L flavonoids and 0.43 ± 0.06 mg TAE/L tannins, and exhibiting high antioxidant capacity, saving simultaneously time and energy, compared with other conditions analyzed. Two other aspects of the extraction process studied that deserve to be highlighted are the fact that the recovery of the solvents used is relatively straightforward, which represents a plus in terms of sustainability, and that it is not necessary to separate silverskin batches according to the variety of coffee bean, since the composition of the extracts is not significantly affected by the composition of the samples (data not shown). This can make the extraction process more expedite.

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